PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 16 JAN 2006
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Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
LBP1004PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/008985	11.08.2004	19.08.2003

International Patent Classification (IPC) or national classification and IPC
G01N31/02

Applicant
LONZA BIOLOGICS PLC. et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.03.2005	13.01.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/008985

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-17	as originally filed
Claims, Numbers	
1-12	received on 21.10.2005 with letter of 21.10.2005
Drawings, Sheets	
1/6-6/6	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/008985

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

1. Concerning section V

The documents referred to below are numbered according to their order of appearance in the international search report (ISR). Unless otherwise indicated, the relevant passages are those indicated therein.

1.1 Novelty (Art. 33(2) PCT) and inventive step (Art. 33(3) PCT)

1.1.1 The present application concerns a method for assaying tropolone. Tropolone is used in serum-free cell culture medium to supply iron to the cells. According to the application (pg. 1, lines 15-18), biopharmaceuticals obtained from cell cultures grown in medium containing tropolone should be tested for trace amounts of the compound. Therefore, a method allowing sufficient resolution of tropolone is required. In order to solve this problem, the application (see pg. 6, line 29) proposes a method comprising a step in which tropolone is complexed with Cu(II).

1.1.2 Tropolone and derivatives thereof are known from the prior art (e.g. D1 and D2). It is also known that these compounds can be used in serum-free cell culture medium to allow for iron uptake (see D3 or D4). Contrary to the view expressed in the application (pg. 4, line 7), the Cu chelating properties of tropolone and its derivatives have been widely described in the literature (see D2, D6 and D7). However, the prior art does not disclose that this property of tropolone could be used in a method of assaying this substance or derivatives thereof, as described in the present claims. In view of this, it seems possible to recognise novelty for claims 1-12.

1.1.3 With regard to inventive step, none of the prior art documents, including those describing the use of tropolone in serum-free cell culture medium to allow for iron uptake in cultures of cells overexpressing therapeutic proteins (see D4, pg. 5-6, bridging paragraph), refers to the need to assay (or remove) tropolone. Considering D3 or D4 as the closest prior art, the objective technical problem is the provision of a method for assaying tropolone from animal cell culture supernatant or a proteinaceous solution containing an enriched product protein. The solution proposed by the application is a method comprising complexing tropolone with Cu(II) ions, separating the tropolone from the protein, and assaying tropolone by RP-HPLC using a mobile phase comprising Cu(II) ions

and an ion-pairing reagent that is more hydrophobic than TFA. The question to be decided is wether the skilled person would have arrived at the method presently claimed, i.e., whether the skilled person would have felt a need to assay tropolone and whether he/she would have done that as described in the present claims.

a) The need to assay tropolone, particularly when the molecule is used in cell cultures for the production of biopharmaceuticals appears obvious, since it is important to provide bio-products as pure as possible in order to prevent deterioration but also to avoid adverse toxic reactions when the products are administered.

b) The skilled man, once aware of the prior art and faced with the technical problem, would realize from D6 (pg. 238) that tropolone binds Cu(II) with very high affinity and that this property has been used in a method for identifying and quantifying tropolone derivatives by HPLC. The Cu(II) ions were incorporated into the mobile phase by adding $CuSO_4$.

As stated above, the skilled person would be motivated to assay tropolone in compositions of bio-pharmaceuticals. RP-HPLC (a routinely used analytical method) would be an obvious choice and, in view of D6, the skilled person would think of using a mobile phase comprising Cu(II) ions, before trying anything else.

The addition of ion-pairing reagents to the mobile phase in order to increase the retention time is a standard practice in reverse-phase HPLC and, as acknowledged on pg. 5 (lines 10-13), TFA is considered a gold standard. Therefore, it remains to be determined wether it would have been obvious to the skilled man to use an ion-pairing reagent in combination with Cu(II) complexation and whether it would have been obvious to choose an ion pairing reagent more hydrophobic than TFA.

Given the fact that D6 does not refer to the use of an ion-pairing reagent in combination with Cu(II) in the mobile phase, it would appear that this step would imply inventive merit, particularly the choice of an ion-pairing reagent more hydrophobic that TFA. It seems therefore possible to recognise inventive merit for the present claims, in view of the prior art. Nevertheless, the comments under item c (see below) should be borne in mind.

c) From the data provided in the examples, it would appear that not all ion-pair reagents more hydrophobic than TFA can solve the technical problem. In fact, it appears that MSA, even when used in combination with 10% acetonitrile (said to be the most suitable conditions), still results in significant peak tailing (pg. 15, first paragraph and Fig. 1c). Similarly, HSA (more hydrophobic that MSA) only achieves a satisfactory separation of tropolone from the mobile phase peak when used at a concentration of 0.3% in 10% acetonitrile; at 0,1% HSA, tropolone is said to have eluted close to a mobile phase peak and the two peaks did not demonstrate baseline resolution (pg. 15, second paragraph). These observations lend support to the argument that the technical problem is not solved over the entire scope of claim 1, precluding the recognition of inventive step for the present claims. Inventive merit may eventually be recognised, in case it can be shown that "substantially all" (i.e., a significant number of all possible) ion-pairing reagents that are more hydrophobic than TFA can solve the technical problem.

2. Insufficient disclosure (Art. 5 PCT) and support (Art. 6 PCT)

Since it is not clear which ion-pairing reagents more hydrophobic than TFA, from the plethora of all possible ones, can solve the technical problem, the subject-matter of claim 1 is considered to be insufficiently disclosed and supported. In this regard, attention is drawn to the fact that, given the observations on pg. 15 (see above, item 1c), it is highly unlikely that all the ion-pairing reagents more hydrophobic than TFA can solve the technical problem. Therefore, the skilled person would be faced with undue burden and would need inventive skill in order to determine which ion-pairing reagent would be a suitable solution.

3. Concerning section VI

D5 (WO 2004/009823) could be found relevant during the national phase.